

Middle East Respiratory Syndrome Coronavirus in Dromedary Camel Herd, Saudi Arabia

Technical Appendix

Technical Appendix Table. Testing of dromedary camels by RT-PCR and serologic testing for MERS-CoV, Al-Hasa, Saudi Arabia*

				RT-PCR result						
Farm, sampling date	Camel no.	Calf/Adult†	Age	Nasal sample	Oral sample	Fecal sample	Copy/mL‡	Antibody titers§		
Farm A										
2013 Nov 30	1	Adult	13 y	Pos¶	Neg	Neg	2.61 × 10 ⁷	>5,120		
	2	Adult	12 y	—	—	Neg		>5,120		
	3	Adult	10 y	—	Neg	Neg		>5,120		
2013 Dec 4	4	Adult	14 y	—	—	Neg	1.30 × 10 ⁸	>5,120		
	5	Adult	8 y	—	Neg	Neg		640		
	6	Adult	9 y	—	Neg	Neg		2,560		
	2	Adult	10 y	—	Neg	—		>5,120		
	7Calf	Calf	1–2 y	—	Neg	Neg		1,280		
	7Dam	Adult	9.5 y	—	Neg	—		2,560		
	8	Adult	7 y	—	Neg	Neg		1,280		
	9	Adult	6 y	—	Neg	Neg		1,280		
	10	Adult	8 y	—	Neg	Neg		640		
	11Calf	Calf	1–2 y	—	Neg	Neg		—		
	11Dam	Adult	—	—	Neg	Neg		—		
	12	Adult	12 y	—	Neg	Neg		320		
2013 Dec 30	13	Calf	1 y	Pos¶#	—	—	1.78 × 10 ⁸	<20		
	14	Calf	1 y	Pos#	—	Neg		<20		
	15	Calf	1 y	Pos	—	Neg		20		
	16	Calf	1 y	Pos	—	Neg		>5,120		
	17	Calf	40 d	Pos	—	Neg		80		
	18	Calf	40 d	Neg	Neg	—		—		
	19Calf	Calf	1 y	Pos	—	Neg		—		
	19Dam	Adult	—	Neg	—	Pos¶#		—		
	20	Adult	8 y	Neg	—	Neg		>5,120		
	21	Adult	7 y	Pos	—	Neg		320		
	22	Calf	2 wk	Pos	—	Neg		1,280		
	2014 Feb 14	26	Calf	9 mo	Neg	—		Neg	3.38 × 10 ³	>5,120
13		Calf	1 y	Neg	—	Neg	640			
27		Calf	10 mo	Neg	—	Neg	40			
15		Calf	1 y	Neg	—	Neg	160			
17		Calf	3 mo	Neg	—	Neg	1,280			
11Dam		Adult	12 y	Neg	—	Neg	1,280			
19Calf		Calf	1 y	Neg	—	Neg	320			
28Calf		Calf	3 mo	Neg	—	Neg	20			
28Dam		Adult	10 y	Neg	—	Neg	1,280			
Farm B, 2014 Feb 11										
23Calf		Calf	2.5 mo	Neg	—	Neg	—			
23Dam		Adult	7 y	Neg	—	Neg	>5120			
24Calf	Calf	2 mo	Neg	—	Neg	—				
24Dam	Adult	6 y	Neg	—	Neg	1,280				
25Calf	Calf	2 mo	Neg	—	Neg	—				
25Dam	Adult	6 y	Neg	—	Neg	640				

*RT-PCR, reverse transcription PCR; MERS-CoV, Middle East respiratory syndrome coronavirus; Pos, positive; Neg, negative; —, specimen not collected or age information not available.

†Calf defined as dromedary camel <2 y of age; adult defined as dromedary camel ≥2 y of age.

‡Data deduced from the upstream of E assay.

§Pseudotype neutralization antibody titers.

¶Full genome sequenced.

#Virus isolated.

Methods

PCR

Hydrolysis probe-based real-time PCRs targeting upstream of E gene (UpE) and open reading frame (ORF) 1a were used as recommended by the World Health Organization (WHO). We also tested each specimen with broad-range reverse transcription PCR (RT-PCR) conserved across the coronavirus family to detect other coronaviruses. The nucleic acid extraction methods, primers, and PCR testing protocols used have been described (1).

In brief, nucleic acid was extracted from 140- μ L aliquots of swab supernatants using QIAamp Viral RNA Minikit (QIAGEN, Hilden, Germany) following the procedure recommended by the manufacturer. Viral RNA was eluted in 60 μ L elution buffer provided in the kit, and 12 μ L was used for preparing a 20- μ L reverse transcription reaction with Superscript III reverse transcriptase (Life Technologies, Carlsbad, CA, USA). Complementary DNA (cDNA) produced was subjected to the detection of Middle East respiratory syndrome coronavirus (MERS-CoV) by using probe-based real-time PCRs targeting UpE and ORF1a as recommended by WHO. For the upE qPCR, a 15- μ L reaction was set up containing 1 μ M of each of the forward (5'-GCAACGCGCGATTCAGTT-3') and reverse (5'-GCCTCTACACGGGACCCATA-3') primers, 0.5 μ M probe (5'-FAM/CTCTTCACATAATCGCCCCGAGCTCG/3'-TAMSp), and 2 μ L cDNA in 1 \times reaction buffer (Takara, Kyoto, Japan). The reaction was carried out in ViiA 7 real-time PCR system (Life Technologies) with 40 cycles of amplification. For the ORF1a quantitative PCR, the reaction was set up as above except with forward primer (5'-CCACTACTCCCATTTTCGTCAG-3'), reverse primer (5'-CAGTATGTGTAGTGCGCATATAAGCA-3'), and probe (5'-FAM/TTGCAAATTGGCTTGCCCCCACT/3'-TAMSp) targeting the ORF1a gene. Cycle threshold was generated by the ViiA7 system automatically with default settings.

The full genome of MERS-CoV in dromedary camel swab samples was deduced by sequencing PCR amplicons with overlapping sequence reads. Genome sequence of the virus was constructed by concatenating the amplicon sequences obtained. The sequencing was done with 3–4 times coverage. Genes of the dromedary MERS-CoV was identified by ORF prediction and with reference to published human MERS-CoV genomes. Dromedary MERS-CoV genomes were aligned with human MERS-CoV genome sequences retrieved from

GenBank. Phylogenetic trees were constructed by using MEGA5 with neighbor-joining and bootstrap resampling (2).

Virus Isolation

A 200- μ L aliquot of original sample was filtered through 0.4- μ m filters (Millipore, Billerica, MA, USA) and 100 μ L aliquots of neat 1:10 diluted and 1:100 diluted samples were inoculated into Vero E6 cell (ATCC CRL-1586) in T25 flasks with \approx 80% confluence. The inoculated cells were kept in minimum essential medium without fetal bovine serum and incubated at 37°C for 6 days. The cells were examined daily to detect virus cytopathic effect (CPE). Upon appearance of CPE, or at day 6 if no CPE was observed, the cells were harvested and passaged for a second time in Vero E6 cells. The cells and supernatant of flasks showing CPE was harvested, aliquoted, and stored at -80°C .

Serology

HIV pseudoparticles bearing the MERS-CoV spike protein was prepared as described (3). HIV/MERS pseudoparticles (5 ng of p24) were pre-incubated with serially diluted heat inactivated serum for 30 min at 4°C and then added to Vero E6 cells (ATCC CRL-1586) in triplicate in 96-well microtiter plates. Residual virus replication was assayed at 2 days post infection as described (3). The highest serum dilution giving a 90% reduction of luciferase activity was regarded as the ppNT antibody titer.

References

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